CLAIMS

WE CLAIM:

1. A bacterium having a genome that is genetically engineered to be at least 5% smaller than the genome of its native parent strain.

2. The bacterium of Claim 1, wherein its genome is genetically engineered to be at least 8% smaller than the genome of its native parent strain and wherein there are no scars in the genome resulting from the deletions.

3. The bacterium of Claim 1, wherein its genome is genetically engineered to be at least 14% smaller than the genome of its native parent strain.

4. The bacterium of Claim 1, wherein its native parent strain is an *E. coli* strain.

5. The bacterium of Claim 1, wherein the genome of the bacterium is genetically engineered to delete from the genome of the bacteria DNA selected from the group consisting of flagella gene, a restriction modification system gene, a lipopolysaccharide surface synthetic gene, an insertion sequence element, a rhs element, a non-transcribed region in the genome of the native parent strain and a gene or other DNA sequence that is not present in both of any two bacteria strains of the same species of the native parent strain.

6. An *E. coli* bacterium having a genome that is genetically engineered to be smaller than 4.27 Mb.

7. A strain of *E. coli* bacteria having a genome is genetically altered to be smaller than 4.00 Mb.

8. A method for making a deletion in the genome of a bacterium at a selected genome region of known sequence without introducing scars, the method comprising the steps of:

making an artificial DNA sequence, the artificial DNA sequence comprising: on one end a sequence number one identical to a genome sequence on the left flank of the genome region to be deleted, followed by a sequence number two identical to a genome sequence on the right flank of the genome region to be deleted; on the other end a sequence number three identical to a genome sequence within the genome region to be deleted; and a sequence-specific nuclease recognition site between the sequence numbers one and two on one end of the linear DNA molecule, and the sequence number three on the other end of the linear DNA molecule, the recognition site is not present in the genome of the bacterium;

introducing the artificial DNA sequence into the bacteria under conditions favoring homologous recombination between the first and third sequences and sequences in the genome of the bacteria;

introducing into the bacterium whose genome contains the correct site insertion of the linear DNA molecule an expression vector for a sequence-specific nuclease that recognizes the recognition site;

expressing the sequence-specific nuclease in the bacteria; and

collecting the bacterium that survives the expression of the sequence-specific nuclease and contains the correct deletion.

9. The method of Claim 8, wherein the artificial DNA sequence is introduced into the bacterium by a vector carrying the artificial DNA sequence.

10. The method of Claim 8, wherein the artificial DNA sequence is introduced into the bacterium directly.

11. The method of Claim 8, wherein the artificial DNA sequence further comprises:

a selectable marker gene between the sequence numbers one and two on one end of the artificial DNA sequence, and the sequence number three on the other end of the artificial DNA sequence.

12. The method of Claim 8, wherein the sequence-specific nuclease is I-SceI.

13. The method of Claim 8 further comprising a system that can increase the frequency of homologous recombination between the linear DNA molecule and the genome of the bacterium, the system being the phage Red recombinase system.

14. A method for making a deletion of DNA of known sequence from the genome of a bacterium without introducing scars comprising the steps of:

providing a bacterium containing an expression vector for a system that can increase the frequency of homologous recombination and an expression vector for a sequence-specific nuclease that recognizes a recognition site which is not present in the genome of the bacterium, the expression of the sequence-specific nuclease is under the control of an inducible promoter and the two expression vectors are compatible;

introducing an artificial DNA sequence into the bacterium, the artificial DNA sequence comprising: on one end a sequence number one identical to a genome sequence on the left flank of the genome region to be deleted, followed by a sequence number two identical to a genome sequence on the right flank of the genome region to be deleted; on the other end a sequence number three identical to a genome sequence within the genome region to be deleted; and a recognition site between the sequence numbers one and two on one end of the linear DNA molecule, and the sequence number three on the other end of the linear DNA molecule, the recognition site not present in the native genome of the bacterium;

expressing the sequence-specific nuclease; and

collecting the bacterium that contains the correct deletion.

15. The method of Claim 14, wherein the linear DNA molecule further comprising:

a selectable marker gene between the sequence numbers one and two on one end of the artificial DNA, and the sequence number three on the other end of the artificial DNA.

16. The method of Claim 14, wherein the system that can increase the frequency of homologous recombination is the phage λ Red recombinase system.

17. A method for making a deletion in the genome of a bacterium at a selected genome region of known sequence without introducing a scar, the method comprising the steps of:

providing a vector that comprises a tetracycline promoter, a lambda origin of replication controlled by the tetracycline promoter, and an antibiotic resistant gene;

inserting a DNA insert into the vector, the DNA insert comprises two DNA sequences located next to each other wherein one DNA sequence is identical to a sequence that flanks the bacterial genome region to be deleted on one side and the other DNA sequence is identical to a sequence that flanks the bacterial genome region on the other side;

transforming bacteria with the vector containing the DNA insert;

inactivating the tetracycline promoter and exposing the bacteria to the antibiotic to select for bacteria in which the vector has integrated into the bacterial genome;

activating the tetracycline promoter in the bacteria in which the vector has integrated into the genome; and

identifying bacteria in which the genome region to be deleted has been deleted.

18. The method of Claim 17, wherein activating and inactivating the tetracycline promoter is achieved through adding and removing a tetracycline inducer.

19. A method for making a deletion in the genome of a bacterium at a selected genome region of known sequence without introducing a scar, the method comprising the steps of:

providing a vector that comprises a tetracycline promoter, a lambda origin of replication controlled by the tetracycline promoter, an antibiotic resistant gene, and a sequence-specific nuclease recognition site;

inserting a DNA insert into the vector, the DNA insert comprises two DNA sequences located next to each other wherein one DNA sequence is identical to a sequence that flanks the bacterial genome region to be deleted on one side and the other DNA sequence is identical to a sequence that flanks the bacterial genome region on the other side;

transforming bacteria with the vector containing the DNA insert;

inactivating the tetracycline promoter and exposing the bacteria to the antibiotic to select for bacteria in which the vector has integrated into the bacterial genome;

introducing into the bacteria in which the vector has integrated into the bacterial genome an expression vector for a sequence-specific nuclease that recognizes the recognition site;

expressing the sequence-specific nuclease in the bacteria; and

identifying bacteria in which the genome region to be deleted has been deleted.

20. The method of Claim 19, wherein the sequence-specific nuclease is I-SceI.

21. A method for replacing a selected region of a bacterial genome with a DNA sequence wherein the DNA sequence and the selected region can undergo homologous recombination, the method comprising the steps of:

providing a vector that comprises a tetracycline promoter, a lamda origin of replication controlled by the tetracycline promoter, and an antibiotic resistant gene;

inserting a DNA insert into the vector, the DNA insert comprises the DNA sequence for replacing the selected region of the bacterial genome;

transforming bacteria with the vector containing the DNA insert;

inactivating the tetracycline promoter and exposing the bacteria to the antibiotic to select for bacteria in which the vector has integrated into the bacterial genome;

activating the tetracycline promoter in the bacteria in which the vector has integrated into the genome; and

identifying bacteria in which the selected genome region has been replaced with the DNA sequence.

22. The method of Claim 21, wherein activating and inactivating the tetracycline promoter is achieved through adding and removing a tetracycline inducer.

23. A method for replacing a selected region of a bacterial genome with a DNA sequence wherein the DNA sequence and the selected region can undergo homologous recombination, the method comprising the steps of:

providing a vector that comprises a tetracycline promoter, a lambda origin of replication controlled by the tetracycline promoter, an antibiotic resistant gene, and a sequence-specific nuclease recognition site;

inserting a DNA insert into the vector, the DNA insert comprises the DNA sequence for replacing the selected region of the bacterial genome;

transforming bacteria with the vector containing the DNA insert;

inactivating the tetracycline promoter and exposing the bacteria to the antibiotic to select for bacteria in which the vector has integrated into the bacterial genome;

introducing into the bacteria in which the vector has integrated into the bacterial genome an expression vector for a sequence-specific nuclease that recognizes the recognition site;

expressing the sequence-specific nuclease in the bacteria; and

identifying bacteria in which the selected genome region has been replaced with the DNA sequence.

24. The method of Claim 23, wherein the sequence-specific nuclease is I-SceI.

25. A method for making a deletion in the genome of a bacterium at a selected genome region of known sequence, the method comprising the steps of:

providing a vector that comprises a sequence-specific nuclease recognition site and two DNA sequences one of which is identical to a sequence that flanks a bacterial genome region to be deleted on one side and the other of which is identical to a sequence that flanks the bacterial genome region on the other side, wherein the two DNA sequences are located next to each other on the vector and wherein the sequence-specific nuclease recognition site is located outside the two DNA sequences on the vector;

introducing the vector into bacteria;

introducing into the same bacteria an expression vector for a sequence-specific nuclease that recognizes the recognition site;

introducing into the same bacteria a system that can increase the frequency of homologous recombination and at the same time expressing the sequence-specific nuclease in the bacteria; and

identifying bacteria in which the genome region to be deleted has been deleted.

26. The method of Claim 25, wherein the sequence-specific nuclease is I-SceI.

27. The method of Claim 25, wherein the system that can increase the frequency of homologous recombination is the phage λ Red recombinase system.

28. A method for replacing a selected region of a bacterial genome with a DNA sequence wherein the DNA sequence and the selected region can undergo homologous recombination, the method comprising the steps of:

providing a vector that comprises a sequence-specific nuclease recognition site and the DNA sequence for replacing the selected region of the bacterial genome, wherein the sequence-specific nuclease recognition site is located outside the DNA sequence on the vector;

introducing the vector into bacteria;

introducing into the same bacteria a system that can increase the frequency of homologous recombination;

introducing into the same bacteria an expression vector for a sequence-specific nuclease that recognizes the recognition site;

expressing the sequence-specific nuclease in the bacteria; and

identifying bacteria in which the genome region to be replaced has been replaced.

29. The method of Claim 28, wherein the sequence-specific nuclease is I-SceI.

30. The method of Claim 28, wherein the system that can increase the frequency of homologous recombination is the phage λ Red recombinase system.